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Nile red membrane staining

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An abbreviated version of this protocol was published in Scientific Reports in Apr 2021

Anandamide alters the membrane properties, halts the cell division and prevents drug efflux in multidrug resistant Staphylococcus aureus

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Detailed protocol

Nile red combined with DAPI staining of *Staphylococcus aureus* in suspension

1. The day before experiment, inoculate a frozen stock of *Staphylococcus aureus* 1:100 in TSB (e.g., 100 microliter bacteria in 10 ml TSB), and incubate at 37^{o}C for 16 h.
2. Dilute the overnight culture to an initial OD_{600nm} of 0.1 in TSB containing 1% D-glucose (TSBG) and incubate at 37^{o}C until reaching an OD_{600nm} of 0.3.
3. Treat your bacteria (*Staphylococcus aureus*) with your compounds in TSBG for desired time periods.
4. At the end of the incubation, add Nile red to a final concentration of 10 microgram/ml and DAPI to a final concentration of 1 microgram/ml and incubate for 30 min at 37^{o}C. You can make a x10 solution of Nile Red and DAPI in TSBG (100 microgram/ml Nile Red and 10 microgram/ml DAPI) and add 100 microliter of this mixture to each 900 microliter of bacteria.
5. At the end of the staining procedure, centrifuge the bacteria at 5,000 x g for 5 min at 4^{o}C, and wash them once with PBS, and recentrifuge them.
6. Resuspend the bacterial pellet in a small volume of PBS (20-50 microliter), and then add 1 ml of 1% paraformaldehyde in ddw to fix the bacteria for 30 min at room temperature. It is important to resuspend the bacteria well with a thin tip to avoid clumping, before adding the fixative.
7. Recentrifuge the bacteria, and then resuspend the bacteria in a small volume of PBS (e.g., 10-50 microliter PBS).
8. Place a 22x22mm agarose pad made from 250 microliter 1% agarose in ddw on a pre-labeled slide.
9. Immediately add 2 microliter of the bacterial suspension to the middle of the agarose pad and cover it with a thin 22x22 mm coverslip (no 0).
10. Visualize the stained bacteria under a confocal microscope using the x100 objective and the 561nm excitation laser for nile red and 405nm excitation laser for DAPI, turning the thin coverslip towards the x100 lens that has got a drop of immersion oil.
11. The images can be captured and analyzed by using the NIS element software.

A 10 mg/ml stock solution of Nile red can be prepared in DMSO.
A 1 mg/ml stock solution of DAPI can be prepared in sterile ddw. DAPI is insoluble in PBS.

Related files

Nile red combined with DAPI staining of Staphylococcus aureus in suspension.docx

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Sionov, R. (2022). Nile red membrane staining. Bio-protocol Preprint. bio-protocol.org/prep1585.
2. Banerjee, S., Sionov, R. V., Feldman, M., Smoum, R., Mechoulam, R. and Steinberg, D.(2021). Anandamide alters the membrane properties, halts the cell division and prevents drug efflux in multidrug resistant Staphylococcus aureus. Scientific Reports 0(0). DOI: 10.1038/s41598-021-88099-6